Transposome assembly using Diagenode Tagmentase

PROTOCOL

1. Order the oligos that you would like to use to load the tagmentase. You will need 3 oligos that we can call A, B and Rev. They should be lyophilized
2. Prepare the following Annealing Buffer: 40mM Tris-HCl (pH8.0), 50mM NaCl
3. Resuspend the oligos in Annealing Buffer to stock concentration of 100 µM
4. In a PCR tube, mix 5 µl of oligo Rev with 10 µl of oligo A
5. In a separate PCR tube, mix 5 µl of oligo Rev with 10 µl of oligo B
6. Vortex and place PCR tubes in a thermocycler
7. Run the following programme:

Temperature	Time
95°C	5 minutes
Cool to 65°C	-0.1°C/second
65°C	5 minutes
Cool to 4°C	-0.1°C/second

Note: Annealed linker oligos can be stored at -20°C.
You can therefore prepare a bigger volume and freeze it.

8. In a chilled PCR tube, mix 6.25 µl of the annealed oligo A/oligo Rev with 6.25 µl the annealed oligo B/oligo Rev
9. Add 10 µl of Diagenode Tagmentase (Cat. No. C01070010)
10. Vortex briefly and incubate at 23°C for 30 minutes in a thermocycler

Caution: *Do not exceed 60 minutes incubation time, or the Tagmentase will lose activity*

11. If not for immediate use, add 12.5 µl of glycerol and store at -20°C

Note: If dilution of the transposome is needed we recommend using the Tagmentase Dilution Buffer which is available separately (Cat. No. C01070011). This buffer contains 50% glycerol

REFERENCE

Picelli S, Björklund AK, Reinius B, Sagasser S, Winberg G, Sandberg R. Tn5 transposase and tagmentation procedures for massively scaled sequencing projects. *Genome Res.* 2014;24(12):2033–2040. doi:10.1101/gr.177881.114

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